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(54) Title: ANALYTICAL METHOD AND KIT

(57) Abstract: Analytical methods using RNA probes for the detection or analysis of nucleic acid sequences is described. These probes are contacted with a sample suspected of containing the nucleic acid sequence and if they form duplexes, they are hydrolysed. This may be done, for example during an amplification reaction. AMP generated as a result of the hydrolysis is converted to ATP. The ATP may then be detected using bioluminescent reagents.

# INTERNATIONAL SEARCH REPORT

Int...onal Application No
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A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, MEDLINE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 13 June 2003 | 27.08.03 |

| Name and mailing address of the ISA | Authorized officer |
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| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax: (+31-70) 340-3016 | Mabit, H |

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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| Y | EP 0 639 647 A (TANABE SEIYAKU CO ;EIKEN CHEMICAL (JP)) 22 February 1995 (1995-02-22) claim 1 --- | 1-25,34, 35 |
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| Y | US 4 735 897 A (KOUDELKA ASTRID P ET AL) 5 April 1988 (1988-04-05) column 1, line 59 -column 2, line 18 ----- | 1-25,34, 35 |

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 02/02096

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-25, 34-35

**Remark on Protest**

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-25, 34-35

a. A method for detecting or analysing a nucleic acid sequence in a sample, said method comprising contacting said sequence with an RNA probe under conditions such that the probe will bind to the sequence, subjecting any nucleic acid/probe complex to conditions under which RNA probe bound to nucleic acid is hydrolysed to generate AMP, detecting AMP produced, and relating this to the presence or nature of the nucleic acid sequence in the sample.
b. A method for detecting the presence or amount of a target nucleic acid within a sample, said method comprising conducting an amplification reaction in the presence of (a) an RNA probe which is specific for at least a portion of said target nucleic acid, (b) an enzyme which hydrolyzed RNA when in double stranded form and (c) one or more enzymes or reagents necessary to convert AMP produced to ATP, adding to the sample bioluminescent reagents which react to the presence of ATP, detecting a signal from said bioluminescent reagents and relating that to the presence or amount of the target nucleic acid sequence.
c. A method for determining the sequence of a nucleic acid, said method comprising (i) binding an RNA probe to a known region of said sequence such that at least one nucleotide at an end of said probe reaches into an unknown or uncertain region of the sequence, (ii) hydrolysing the RNA probe using an enzyme which hydrolyses RNA only when in double stranded form, (iii) converting AMP produced to ATP, (iv) adding to the sample bioluminescent reagents which react to the presence of ATP, (v) detecting a signal from said bioluminescent reagents, and (vi) relating that signal to the presence of a region of the sequence which is complementary or otherwise to the end of the probe.
d. A method for detecting the presence or amount of a target nucleic acid within a sample, said method comprising denaturing nucleic acids within a sample, contacting these with an RNA hydrolysis probe which is specific for at least a portion of said target nucleic acid so that the probe forms duplexes with the target nucleic acid; adding an enzyme which hydrolyses RNA when in double stranded form and one or more enzymes or reagents necessary to convert AMP produced to ATP; adding to the sample biolumin

2. Claims: 26-33

A kit for use in analysis comprising at least one RNA probe which is specific for a target sequence, and a enzyme which can hydrolyse RNA when in double stranded form.

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International Application No
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